

Clinical, haematological, biochemical and economic impacts of *Trichinella spiralis* infection in pigs

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Abstract

The purpose of this work was to assess the clinical, haematological and biochemical responses of pigs experimentally inoculated with *Trichinella spiralis*. Groups of three pigs were inoculated per os with 100, 500 and 5000 *T. spiralis* muscle larvae, two pigs were used as control. Clinical evaluation of disease in pigs included daily examination, rectal temperature measurements and cardiac and respiration rates. Haematological studies included: hematocrit (%), hemoglobin (g/dl), and white cell, neutrophil, lymphocyte and eosinophil counts. Blood biochemistry included: bun (mg/dl), creatinine (mg/dl), AST (UI/l), ALT (UI/l), CPK (UI/l) and ALP (UI/l). No significant differences were observed in rectal temperature and in cardiac and respiration rates between inoculated animals and the control group ($p \geq 0.05$). Significant differences were detected ($p \leq 0.05$) in the values of % hemoglobin, and eosinophils, as well as in the values of CK, ALP, AST and ALT. The variations observed in some cases were related to the number of *T. spiralis* larvae inoculated and varied with the number of days post-infection. Inoculated pigs showed significant differences ($p \leq 0.05$) in weight gain when compared with uninoculated controls. This study has clinical, haematological, and enzyme alterations in *Trichinella* infected pigs provides a better understanding of acute and chronic trichinellosis in pigs.

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1. Introduction

The genus *Trichinella* is widely distributed throughout the world and is found primarily in carnivorous mammals including humans. It has also been reported in birds and crocodiles (Pozio and La Rosa, 2002). Traditionally, the epidemiology of *Trichinella* in domestic livestock is limited to pigs. However, horses

have been the cause of a number of human outbreaks in France and Italy beginning in 1975 (Pozio et al., 1998; Boireau et al., 2000). It is thought that horses may become infected by the deliberate feeding of meat scraps. In Argentina where *Trichinella* infection in pigs is endemic and pork is a source of human infection, human outbreaks have been linked to meat from wild animals but never horsemeat (Ribicich et al., 2005).

Penetration of *Trichinella* larvae into striated skeletal muscle cells results in ultrastructural and metabolic changes (Despommier, 1998). Migration of larvae causes the typical symptoms and signs of the disease.

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In pigs, establishment of the L₁ of *Trichinella spiralis* results in extensive disturbances at the cellular level and consequent pathological changes in skeletal muscles and organs (Ribicich et al., 2004). The present study was initiated to assess the clinical, haematological and biochemical responses of pigs experimentally inoculated with *T. spiralis*.

2. Materials and methods

2.1. Pig infections

The work was carried out with 11 commercial hybrid pigs, *Landrace* and *Large White* crossbred, originating from a cattle-breeding ranch in the province of Buenos Aires. Pigs were 60 days old at the beginning of the experiment and weighed approximately 20 kg. All animal work was performed in the swine breeding facility belonging to the Faculty of Veterinary Sciences of the University of Buenos Aires. Inoculation of pigs with infective larvae of *T. spiralis* was performed as described in a previous study (Ribicich et al., 2000). The animal groups and numbers of parasites inoculated are listed in Table 1.

2.2. Clinical signs and weight gain

During the first 30 days post-infection, body temperature, cardiac and respiration rates were measured every 48 h. Weight gain was measured at 20-day intervals until 100 days post-inoculation.

2.3. Blood, fecal samples and tissue recovery

Prior to inoculation, and at 7-day intervals thereafter, pigs were bled by jugular venipuncture and blood samples were collected. The serum was stored at –20 °C until used. Fecal samples were collected at 48-h intervals during the first 30 days post-inoculation for the diagnosis of parasites (Telemann, 1908; Willis, 1921; Baermann, 1917). On day 100 post-inoculation, all 11 pigs were sedated and euthanized by exsanguination.

Table 1
Experimental design for infection of pigs with *Trichinella spiralis*

Group	No. of animals	No. of infective <i>T. spiralis</i> larvae	Targeted infection level
0	2	0	Control
1	3	100	Low
2	3	500	Moderate
3	3	5000	High

Following euthanasia, the animals were eviscerated and diaphragm tissue was collected for artificial digestion.

2.4. Enzyme and blood analysis

Blood was preserved with EDTA (0.77%). Packed cell volume (PVC) was determined by the micro-hematocrit method using a Rolco CH24 microcentrifuge. Determination of hemoglobin was by the hemoglobin cyanide method. The relative leukocyte differential count was determined using May-Grunwald Giemsa stain and observed microscopically at 100×. White blood cell (WBC) counts were done in a hemocytometer (Neubauer) using hydrochloric acid at 1% (v/v) in water, as a diluent. Serum creatinine was determined using the creatinine kinetic AA method (Wiener Lab Group) at 500 nm without deproteinization in serum. Serum bun was determined using the Bun enzymatic method (Wiener Lab Group). Alanine transferase (ALT) and aspartate transferase (AST) concentrations in blood were analysed using the Transaminase 200 colorimetric method (Wiener Lab Group). Alkaline phosphatase was determined using an optimized alkaline phosphatase method. Creatine kinase (CK) in serum was analysed using an optimized UV method (CK-NAC UV (Wiener Lab Group)). Measurements were made using a Shimadzu spectrophotometer UV-120-02 and SPR-N Atago refractometer (Raitman and Frankel, 1967; Frankel, 1970; Jain, 1993; Willard et al., 1989).

2.5. Artificial digestion

The presence of *T. spiralis* larvae in pigs was determined by digestion of 100 g of diaphragm tissue. Briefly, samples were weighed, ground in a commercial meat grinder, and mixed with 1 l of artificial digestion fluid (1.0% pepsin [1:10,000 Sigma] and 1.0% hydrochloric acid [v/v]). Digests were mixed vigorously on a magnetic stir plate at 42–44 °C for 2 h. At the conclusion of the 2 h, the digest was allowed to settle and the supernatant was decanted. The sediment was poured through an 80 µm mesh sieve into round-bottom pilsner glasses. Following settling for 20 min, the sediment, containing *T. spiralis* larvae was washed repeatedly in tap water. Recovered larvae were counted using a stereo microscope at a 10× magnification (Gamble et al., 2000).

2.6. Serology testing

The ELISA method was performed as described by Ribicich et al. (2000).

2.7. Statistical analyses

An analysis of variance was performed using a random block design (DBCA) grouping like blocks and like treatments for the days on which the measurement was made. This methodology was used for: body weight, hemoglobin, hematocrit, white cells counts, eosinophil counts and artificial digestion. Comparison of means was performed using the honest significant difference (HSD) Tukey test. The statistical analyses were performed with Statistic (Dawson-Saunders and Trapp, 1997; version 7) software for Windows (Analytical Software, Tallahassee, FL).

3. Results

3.1. Clinical signs

Only two of nine pigs inoculated with *T. spiralis* larvae showed clinical signs consistent with trichinellosis (dyspnea, peri-orbital edema, and respiratory problems). Body temperature and cardiac rates were normal for all pigs, during both the acute and chronic stages of infection.

3.2. Weight gain

Significant differences in weight gains were observed between the control and inoculated groups of pigs ($p \leq 0.05$) (Fig. 1). These changes were not seen until 20 days post-infection. From day 40 to day 100, growth of infected pigs was reduced between 10 and 15%.

3.3. Haematology and biochemistry

In pigs inoculated with 100 larvae of *T. spiralis*, the hematocrit was lower than normal values, with significant differences ($p \leq 0.05$) as compared with

uninoculated controls at 28 days post-infection. Hemoglobin values were decreased in animals inoculated with 500 and 5000 larvae, between 7 and 42 days post-infection. White cell counts were higher between 7 and 42 days in all groups ($p \leq 0.05$) as compared with controls. Lymphocyte counts from groups 2 and 3 showed significant differences ($p \leq 0.05$) between days 21 and 35 post-inoculation and remained higher than control through day 100 post-inoculation. In inoculated pigs eosinophil counts varied with the number of *T. spiralis* larvae; values for inoculated pigs were significantly higher than control pigs ($p \leq 0.05$), but returned to normal by day 42 post-inoculation. Absolute eosinophil counts were as high as 2500 cells/ml in pigs inoculated with 100 larvae, while values ranged from 3000 to 3500 cells/ml in pigs inoculated with 500 and 5000 larvae between days 28 and 35 post-inoculation (Fig. 2). Eosinophil counts were within normal physiologic values in the control group for the duration of the experiment. Renal function, evaluated by analysis of bun and creatinine, was normal in inoculated and uninoculated pigs. CK was considerably elevated in infected pigs between day 28 and day 70 post-inoculation (Fig. 3). AST and ALT were also increased between day 14 and day 70 and levels of these enzymes were related to the number of larvae inoculated (Figs. 4 and 5). ALP values in group 3 pigs showed significant differences from controls ($p \leq 0.05$) between day 28 and day 56 (Fig. 6).

3.4. Parasitological and serological results

Results of artificial digestion of pigs infected with various doses of *T. spiralis* are shown in Table 2. While the low doses given to pigs in this study did not result in larvae being recovered from all inoculated pigs, positive ELISA results for all pigs demonstrate exposure to the parasite.

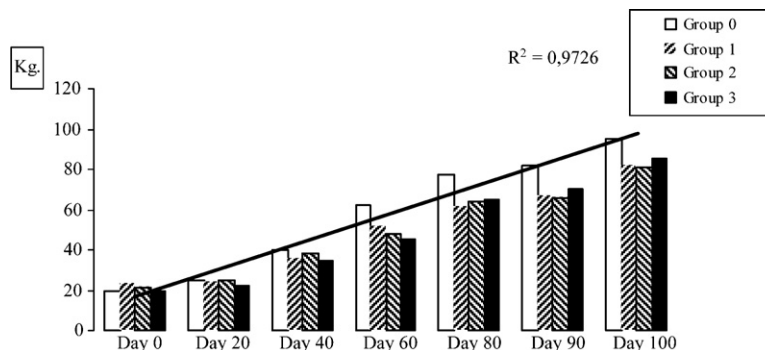


Fig. 1. Weight body in pigs with 100, 500 and 5000 *Trichinella spiralis* infected larvae during 100 days post-infection.

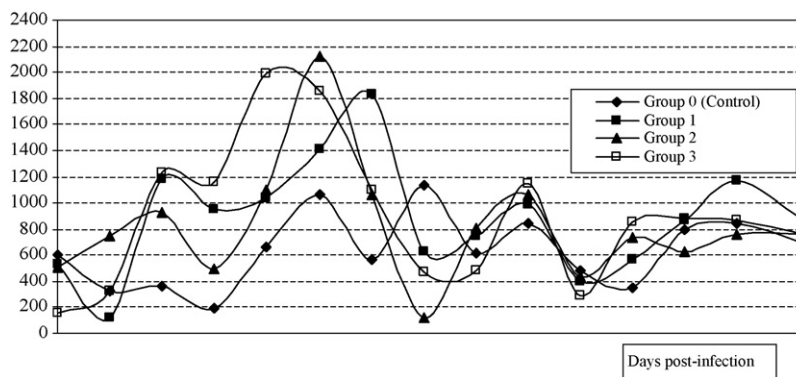


Fig. 2. Absolute count of eosinophils in pigs with 100, 500 and 5000 *Trichinella spiralis* infected larvae during 100 days post-infection.

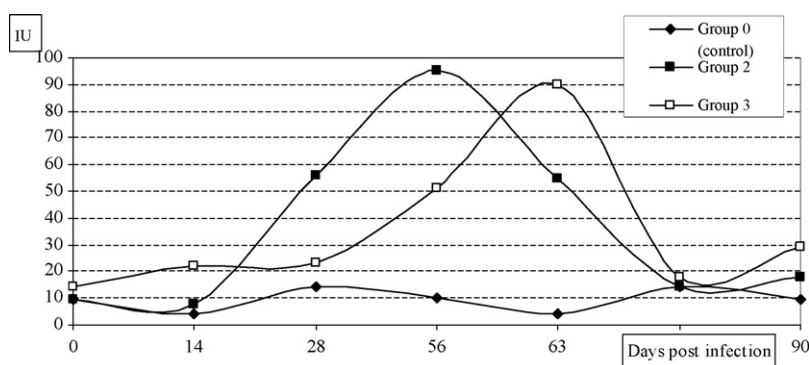


Fig. 3. CK values in pigs absolute count of eosinophils in pigs with 100, 500 and 5000 *Trichinella spiralis* infected larvae during 90 days post-infection.

4. Discussion

In contrast to humans, pigs and horses experimentally inoculated with *T. spiralis* show few signs of disease. In horses the clinical symptoms that have been observed include myalgias and an increase in rectal temperature (Soule et al., 1989). In another study, one horse and one pony experimentally inoculated with *T.*

spiralis showed no signs associated with the infection (van Knapen et al., 1987).

Eosinophilia is a characteristic and consistent sign of human trichinellosis and occurs simultaneously with the common signs and symptoms of disease; as patients recover, eosinophil levels return to normal (Campbell, 1983). Eosinophilia correlates with the intensity of the infection; elevated eosinophil values are detected

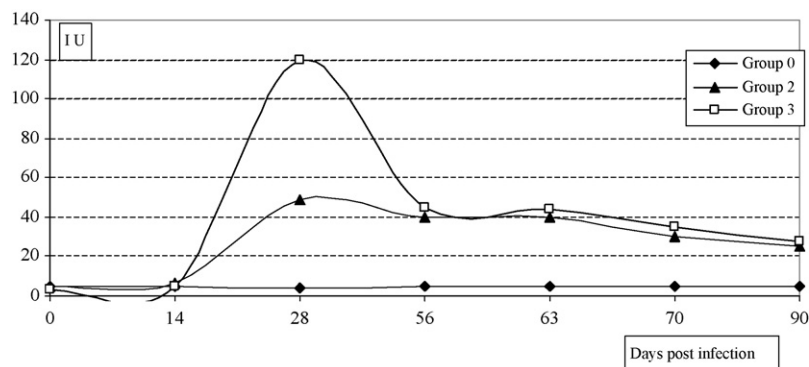


Fig. 4. ALT values in pigs infected with 100 and 5000 *Trichinella spiralis* infected larvae during 90 days post-infection.

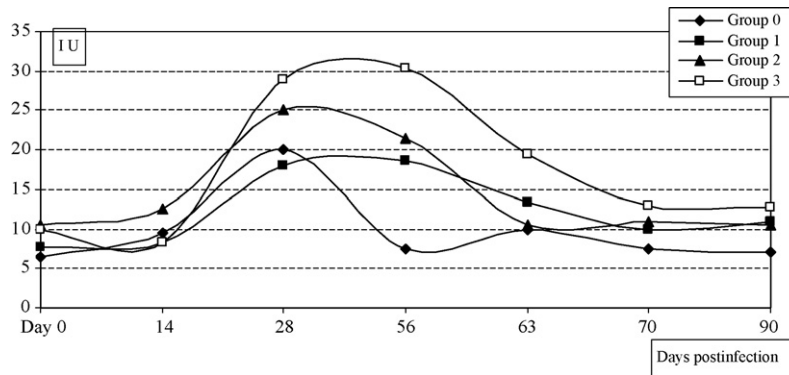


Fig. 5. AST values in pigs infected with 100, 500 and 5000 *Trichinella spiralis* infected larvae during 90 days post-infection.

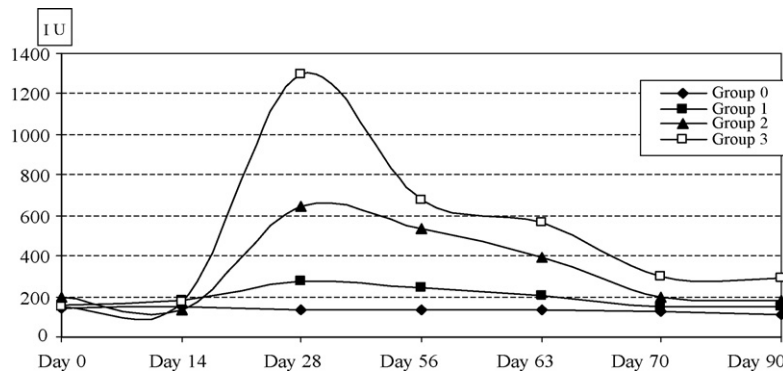


Fig. 6. ALP values in pigs infected with 100, 500 and 5000 *Trichinella spiralis* infected larvae during 90 days post-infection.

between the second and third week following infection. Values reported in human trichinellosis patients range from 1000 to 3000/mm³ (15–45% of the white cells), but in some cases could be higher (Campbell, 1983). In horses, an increase in the number of eosinophils has not been reported to correlate with the number of infective

Table 2
Results of artificial digestion from pigs infected with different doses of *Trichinella spiralis*

Group	Pig no.	No. of larvae/ gram tissue
0	6	0
	7	0
1	9	0
	18	4
	21	2
2	4	20
	13	0
	14	0
3	5	40
	8	72
	15	8

larvae inoculated (Soule et al., 1989). In the present study, we did find a correlation ($p \leq 0.05$) between the number of *T. spiralis* larvae inoculated and the increased level of eosinophils.

Renal function was evaluated here through the analysis of bun and creatinine values and they were found to be within normal physiologic parameters (bun normal value = 10/30 mg/dl; creatinine normal value = 1/2.5 mg/dl). Serum levels of CK and FAS were increased between day 14 and day 70 post-inoculation in pigs that were inoculated with 500 or 5000 larvae. Elevated CK levels persisted until day 63; these elevated levels correlated with the time of arrival of *Trichinella* newborn larvae in the muscle and the development of an inflammatory process. Pathological changes which occur in the muscle include infiltration of eosinophils, lymphocytes, plasma cells and histocytes, extra- and intra-cellular edema and the degeneration of muscle cells (Ribicich et al., 2004). Similar to our findings in pigs, experimentally-infected horses, showed the highest CK levels at 5 weeks after inoculation and returned to normal values at 7 weeks post-inoculation (Soule et al., 1989). In human trichinellosis, CK increases at 18 day of post-infection (Campbell, 1983).

Significant differences ($p \leq 0.05$) were detected in the values of ALT (normal value = 31/58 UI/l) in pigs from groups 2 and 3; variations of ALT levels are typically related to hepatic failure. Increased levels of the muscle enzymes ALT and AST are also detected in cases of muscular dystrophies and myositis like those that occur in acute trichinellosis. Inoculated pigs showed significant differences ($p \leq 0.05$) in weight gain when compared with uninoculated controls (Fig. 1). In infected pigs growth was reduced between 10 and 15%.

The results presented here provide a better understanding of acute and chronic trichinellosis in pigs. While pigs appear to be refractory to severe disease – the classical trichinellosis syndrome as observed in humans – we have demonstrated alterations in haematological parameters and enzymes caused by infection with *Trichinella*. Further, these laboratory findings correlate with slower growth, as compared to non-infected animals.

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